RITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* Antibodies to liposomes, phospholipids, and cholesterol: Implications for autoimmunity, atherosclerosis, and aging		5. TYPE OF REPORT & PERIOD COVERED 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Carl R. Alving		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS Division of Biochemistry Walter Reed Army Institute of Research Washington, DC 20307-5100		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research & Development Command Washington, DC 20307-5100		12. REPORT DATE 13. NUMBER OF PAGES 11
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)* Walter Reed Army Institute of Research Washington, DC 20307-5100		15. SECURITY CLASS. *(of this report)* UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Antibodies, liposomes, phospholipids, cholesterol, autoimmunity

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Reviews biological implications of antibodies to lipids

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

Horizons in Membrane
Biotechnology, pages 41–51
Published 1990 Wiley-Liss, Inc.

ANTIBODIES TO LIPOSOMES, PHOSPHOLIPIDS, AND CHOLESTEROL: IMPLICATIONS FOR AUTOIMMUNITY, ATHEROSCLEROSIS, AND AGING

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INTRODUCTION

It is often stated that lipids are nonimmunogenic; however, this idea is now known to be a false presumption. In 1979 it was discovered that antibodies to membrane lipids such as phosphatidylcholine and cholesterol could be readily induced in rabbits or mice by injecting liposomes containing lipid A (Schuster et al., 1979; reviewed by Alving, 1986 and 1990) (Fig. 1). In order to produce antibodies to liposomes, lipid A, the lipid portion of gram negative bacterial lipopolysaccharide (LPS), apparently plays an essential role as a potent adjuvant. Antibodies to liposomes were not induced by liposomes lacking lipid A.

Polyclonal antibodies having considerable specificity for individual phospholipid constituents of liposomes have now also been developed (Wassef et al., 1989; Banerji and Alving, 1989). Monoclonal antibodies to liposomes and liposomal constituents, particularly phosphatidylinositol phosphate, have been induced by immunizing mice with liposomes containing lipid A, and specificities of the antibodies have been determined (Banerji et al., 1982; Wassef et al., 1984; Alving et al., 1987).

After developing immunoassay procedures (complement-dependent assays and solid-phase immunosorbent assays) for detecting antibodies to lipids, we and others discovered that naturally-occurring autoantibodies to various phospholipids are widespread and occur in most adult animal or human sera (Strejan et al., 1979 and 1981; Alving, 1983

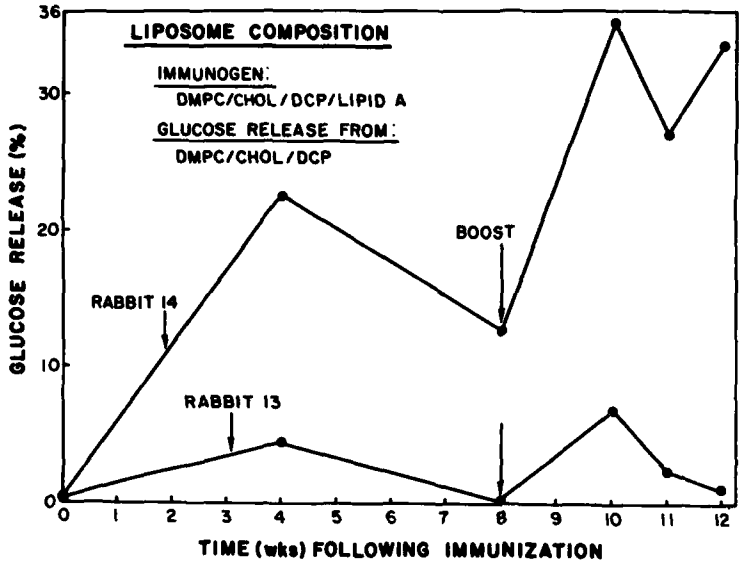

Figure 1. Complement-dependent immune damage to liposomes lacking lipid A after immunization with liposomes containing lipid A. Immune damage was measured by release of trapped liposomal glucose (From Schuster et al., 1979).

and 1984). Fig. 2 demonstrates the observation that naturally-occurring antibodies to phospholipids, which were not present in young mice, did develop in aged mice that were chronically injected with normal saline (Richardson et al., 1988-89).

Antibodies to Cholesterol

In a recent development we have now discovered that murine antibodies are readily produced against cholesterol by injecting cholesterol-laden liposomes containing lipid A (Swartz et al., 1988). Autoantibodies to cholesterol (IgG and IgM) have also been found to occur naturally in nearly all normal human sera (Alving et al., 1989). Recently, it was discovered that pigs have naturally-occurring autoantibodies to cholesterol (Wassef et al., 1989). Upon i.v. injection of liposomes containing cholesterol into pigs, activation of serum complement occurred

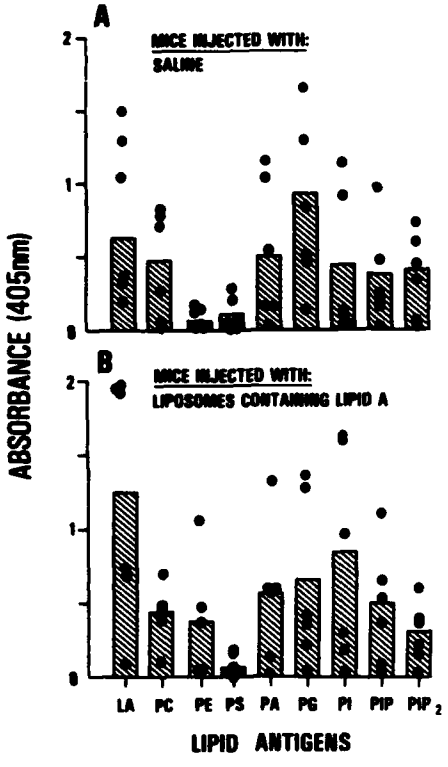

Figure 2. Antibody activities against phospholipids and lipid A in chronically injected mice. Serum from each of six mice surviving at 765 days (25 months) after chronic injection of normal saline (A) or liposomes was assayed by the ELISA method. Antibodies were measured with purified PC, PE, PS, PA, PG, PI, PIP, PIP_2 or lipid A as antigen. Each point is the mean of triplicate samples of a 1/100 dilution of an individual serum expressed as absorbance at 405 nm. Height of column represents the mean activity of the group (from Richardson et al., 1988-89).

and the pigs suffered anaphylactoid reactions characterized by massive eicosanoid secretion and severe pulmonary hypertension (Wassef et al., 1989).

Binding of Antibodies to Phosphates, Nucleotides, and DNA

Antibodies to phospholipids, and even to a slight extent antibodies to cholesterol, also have a subsite in the antigen binding site that binds to soluble phosphorylated compounds such as ATP (Alving, 1986). Because of the phosphate-binding subsite, certain antibodies induced by liposomes containing lipid A bind strongly both to other nucleotides and to denatured DNA (Stollar et al., 1989). Antibodies induced by liposomes containing lipid A therefore apparently share common binding characteristics with certain autoantibodies observed in lupus erythematosus and other autoimmune diseases.

BIOLOGICAL IMPLICATIONS OF ANTIBODIES TO LIPIDS AND LIPID BILAYERS

In past years it was widely believed that most lipids were nonimmunogenic (reviewed by Alving, 1990). However, recent research demonstrating profusions of induced and naturally-occurring antibodies to phospholipids, particularly phosphatidylcholine, has led to development of the concept that antibodies to phospholipids may actually be identical to antibodies secreted by certain types of B lymphocytes (CD5+ cells in humans, Ly-1+ cells in mice). These B cells have been previously associated with autoimmunity in animals (such as NZB mice) and humans (reviewed by van Rooijen, 1989 and Alving, 1990). Autoantibodies that react with the above B cell types are readily detected by the ability to react with erythrocytes treated with a proteolytic enzyme (bromelin) (Linder and Edgington, 1972; Cunningham, 1974; Hayakawa et al., 1984).

It has now been determined by several laboratories that phosphatidylcholine serves as the antigen on bromelin-treated erythrocytes that reacts with the autoantibodies, and the specific epitope that reacts with autoantibodies includes the trimethylammonium group of the choline (Serban et al., 1981; Page et al., 1982; Cox and Hardy, 1985; Mercolino et al., 1986).

As noted earlier for induction of antibodies to liposomes by lipid A, autoantibodies that reacted with bromelin-treated erythrocytes could be induced by the parent component of lipid A, LPS (Fujiwara and Akiyama, 1980; Kawaguchi, 1981 and 1985).

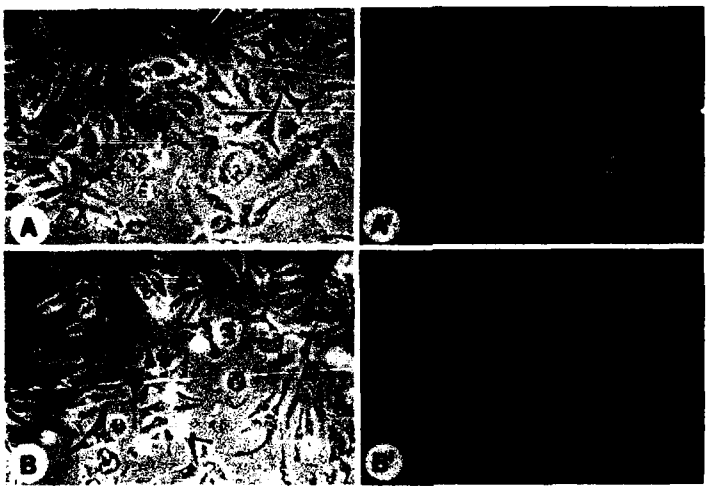

Figure 3. Indirect immunofluorescence detection of macrophage-bound monoclonal antibodies having specificity to liposomes (dimyristoyl phosphatidylcholine/cholesterol/dicetyl phosphate) (A,A') or liposomal PIP (B, B'). Frames A and B are phase contrast micropahges, and A' and B' are immunofluorescence micrographs of the same fields (X350) (from Fogler et al., 1987).

Based on the above observations, it may now be concluded that antibodies to at least one phospholipid (phosphatidylcholine) may play a role in processes of autoimmunity. Moreover, as with induction of antibodies to liposomal phosphatidylcholine (Schuster et al., 1979; Wassef et al., 1989), lipid A (the active portion of LPS) may play an important role for induction of anti-phospholipid antibodies that react in autoimmune mechanisms.

Binding of Antibodies to Cells

I am frequently asked a question that includes some variant of the following: "If naturally-occurring antibodies to lipids are so widespread, why don't we simply disintegrate?" The answer is that we don't disintegrate rapidly because lipids and lipid bilayers are normally

covered with overlying protein that prevents binding of antibodies. This is why cryptic antigen (phosphatidylcholine) on mouse erythrocytes reacts with autoantibodies after treatment of the cells with bromelin. This is also why autoantibodies to cholesterol in pig plasma react with liposomal cholesterol to induce anaphylaxis, but do not react with cholesterol in lipoproteins or plasma membranes (Wassef et al., 1989). In addition to the effects of overlying proteins, large, bulky, or highly charged lipids adjacent to a lipid antigen can also interfere with antibody binding (Shichijo and Alving, 1985 and 1986).

Although under most circumstances antibodies to lipids do not bind to undamaged cells, binding to normal cells can sometimes occur. Monoclonal antibodies to phospholipids did not bind to mouse peritoneal macrophages that were kept in suspension culture, but binding rapidly occurred when the cells were allowed to become adherent to a plastic dish (Fig. 3) (Fogler et al., 1987). Antibody binding to the peritoneal macrophages was enhanced by treatment of cells with trypsin, and was abolished by treatment of cells with phospholipase C.

A Theory of Aging

A further answer to the question "Why don't we disintegrate?" is that all of us <u>are</u> disintegrating slowly. In 1983 I proposed a theory of aging that was based on the experimental observation of induction of autoantibodies to lipids and lipid bilayers by LPS (i.e., lipid A) (Alving 1983) (Fig. 4). According to this theory, autoantibodies to lipids and lipid bilayers are constantly being produced as a result of association of lipid bilayers with lipid A. It was proposed that antibodies against cellular lipid bilayers would be induced exactly in the same way that antibodies against liposomal lipid bilayers are induced. The source of lipid A under natural circumstances would be from the vast amount of LPS and lipid A (endotoxin) that is shed by gram negative bacteria that are present in all parts of the environment to which all living things are exposed.

The amount of endotoxin in the environment is huge, and it is ubiquitiously distributed throughout the world. We even carry an enormous burden of gram negative bacteria in our guts, on our skin, and in air, food and

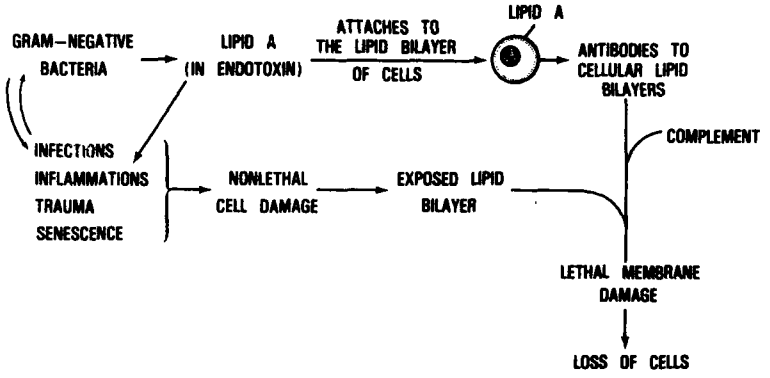

Figure 4. Proposed mechanism of aging (from Alving, 1983).

water, and a certain amount of LPS is consistently absorbed into the blood and detoxified by the liver.

Lipopolysaccharide (lipid A) is known to have a high affinity for binding to lipid bilayers of cells such as erythrocytes or other cells. This property of high affinity association of LPS with erythrocytes is frequently used as the basis of immunohemagglutinin assays for LPS. According to my theory, antibodies are induced against the cellular lipid bilayer to which LPS/lipid A attaches. An expected consequence of this process would be induction of antibodies to lipid A itself, and such antibodies are indeed very widespread in normal human sera (reviewed by Mattsby-Baltzer and Alving, 1984).

Under normal circumstances antibodies to lipids and lipid bilayers cause no harm because the antibodies are sterically hindered from binding to cells for reasons noted above. However, when lipids and lipid bilayers do become exposed for various reasons (Fig. 4), lethal damage may occur due to complement activation (or due to other mechanisms of immunity), resulting in deaths of cells. Amplification of adverse immunologic effects leading to characteristic deficits of aging may occur when losses occur in slow-growing or nonreproducing cells such as endocrine cells, neurons, myocytes, and other important

cells. A particular location that presumably could be affected by antibodies to lipids would be at sites of vascular lesions induced by accumulations of cholesterol, or at sites of local trauma leading to intravascular inflamatory foci.

Accumulations of antibodies and complement at localized sites in the vascular tree would be expected to attract inflammatory cells such as macrophages. Microinflammatory foci would lead to local secretion of mediators such as eicosanoids, serotonin, and histamine. Over a long period of time the above processes might be expected to result in the "wear and tear" appearance that seems to be characteristic of the aging phenomenon.

The above theory was proposed in 1983, but I have recently noted that a remarkably similar theory has been independently proposed by Cox and Hardy (1985) based on studies with bromelin-treated erythrocytes. The following excerpt from the latter authors neatly summarizes the common areas of agreement of both theories.

> "We suggest that autoantibody production against phosphatidycholine may be an example of adaptive immunity against damaged self-components, and propose that in a normal membrane the configuration and/or charge of phosphatidylcholine molecules is such that the autoantibodies do not bind. However if a membrane is damaged, for example by ageing, virus infection, or in the laboratory by proteolysis with enzyme such as bromelain, the autoantibodies recognize the configuration of phosphatidylcholine in the membranes."

In summary, autoantibodies to lipids and lipid bilayers can be induced experimentally by association of LPS or lipid A with liposomes. Naturally-occurring antibodies to lipids and lipid bilayers are very widespread and could reasonably be expected to have been induced by natural association of LPS or lipid A with cellular lipid bilayers. Naturally-occurring antibodies to lipids and lipid bilayers may play an important role in the "wear and tear" processes that occur with ordinary aging. The phenomenon of aging may therefore represent, at least in part, an ultimate and inexorable form of autoimmune disease involving immune reactions with lipid bilayers.

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